Modified Methods in Crystallization

Brookhaven Crystallization Workshop 2007
Focus on Membrane Proteins

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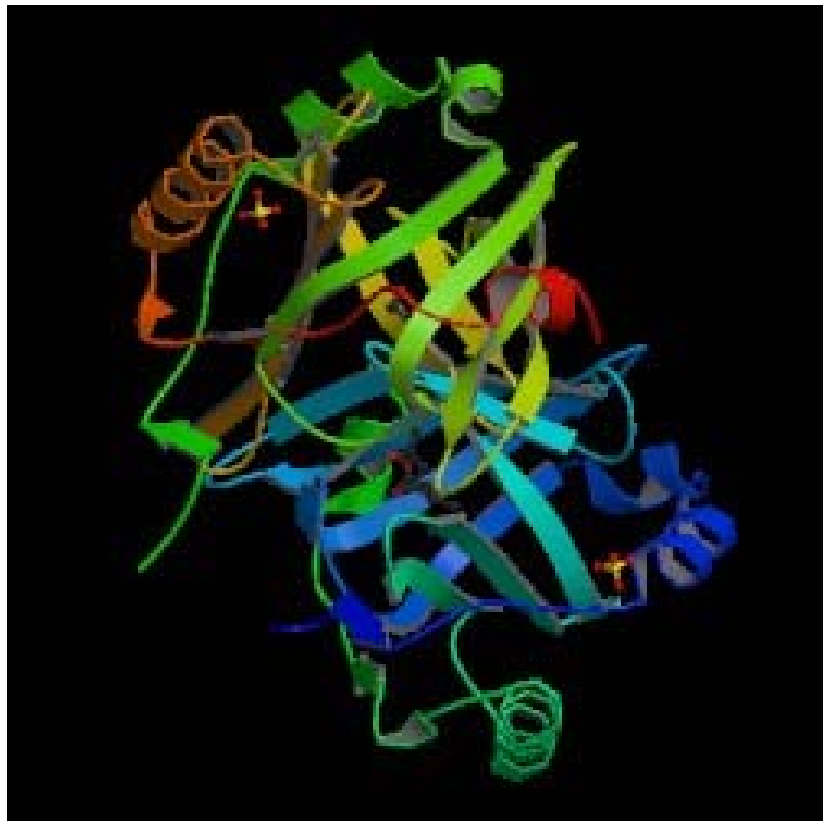
What we are up against

- What makes biological macromolecules so difficult to crystallise?
- Incompatibility between physicochemical factors and the propensity for macromolecules to form regular 3-D arrays
- Other requirements: purity, the requirement for specific ligands or ions, protein-protein contacts etc.

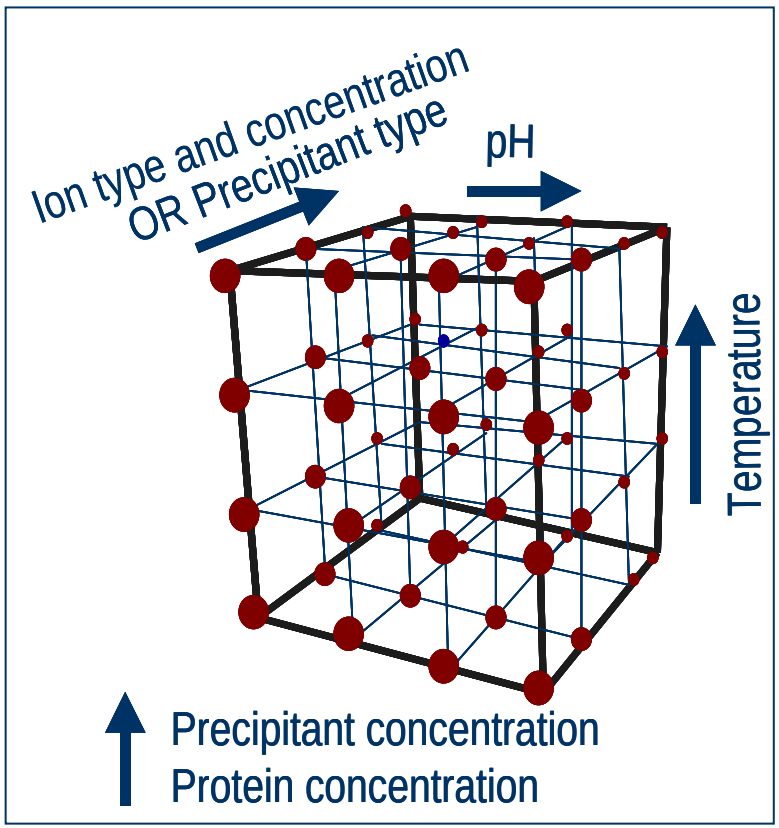


The problem with crystallizing membrane proteins is…

- Membrane proteins exist either almost entirely or partially in a hydrophobic environment. They are made soluble with the aid of detergents
- The major problem is to maintain the protein in a detergent-solublized form that does not disrupt the protein's native conformation and will still allow crystal formation

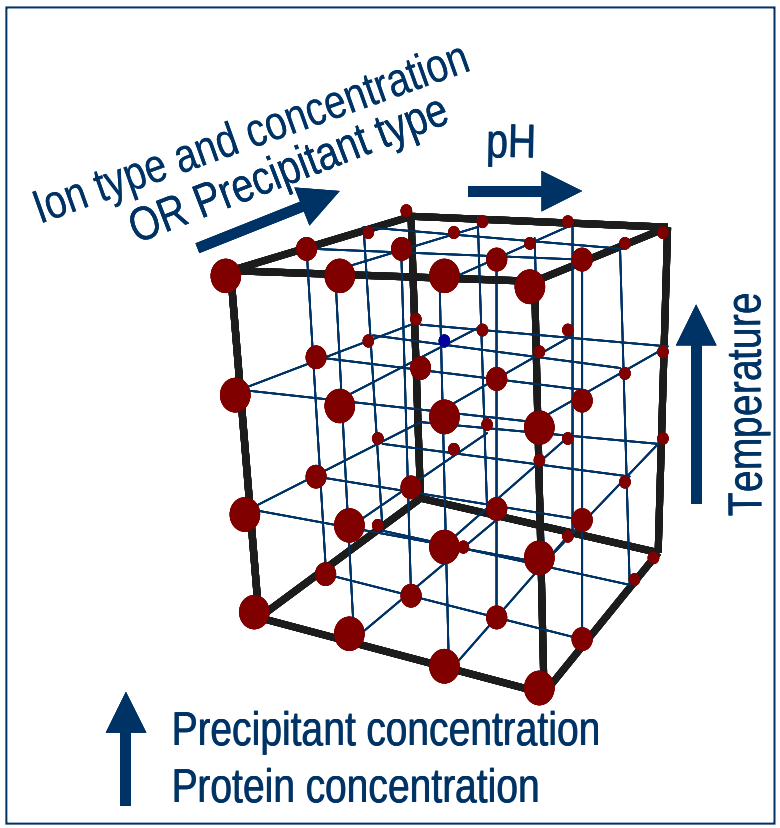
- The levels of complexity are:
  - To determine the appropriate detergent at the optimal concentration for solublization
  - To find conditions that are compatible with the detergent-protein complex that lead to crystallization

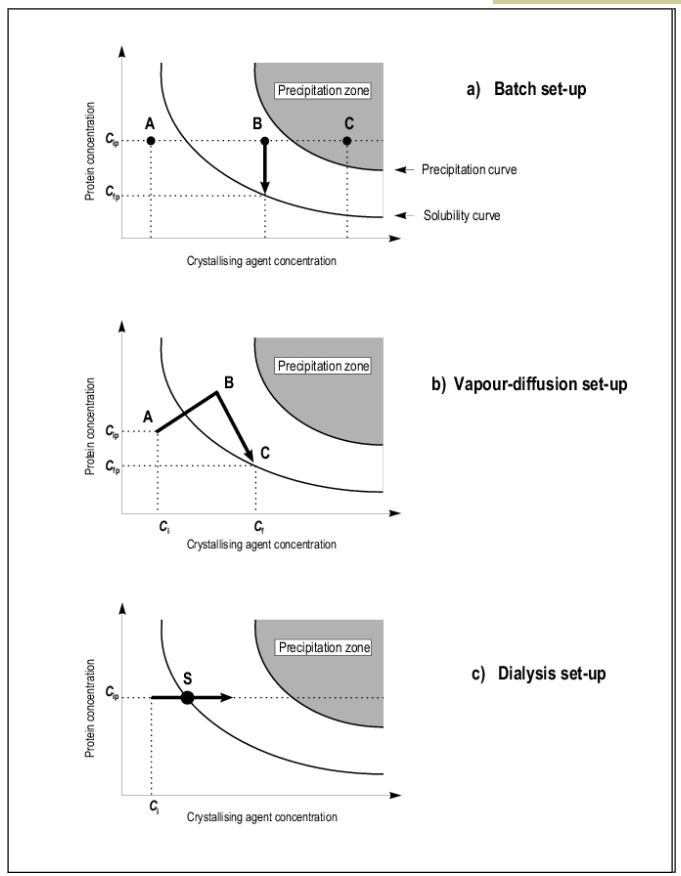
Multi-dimensional space



- It is possible to vary several parameters at the same time to fully explore the multi-dimensional space.
- Sampling finely many 10s of thousands of conditions – high throughput screening
- Sampling a select few conditions – sparse matrix screening

Methods in crystallization

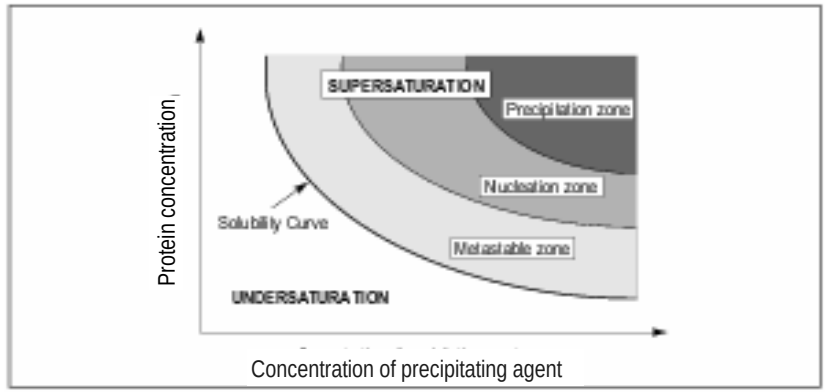


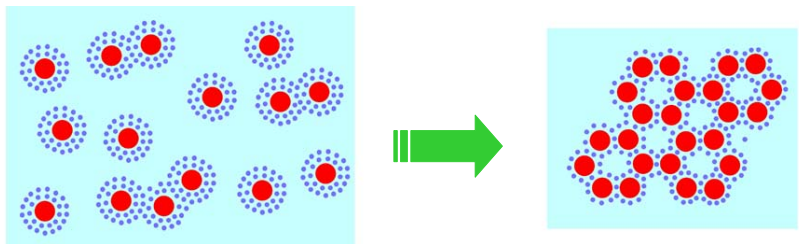


Ducruix, A. and Giege, R. (1992) Crystallization of nucleic acids and proteins. A practical approach, Oxford University Press, New York

Crystallization – step 1

- Achieving nucleation
  - A supersaturated state is required
- Proteins will crystallise from solution if the free energy of the system is lowered as a result
- Supersaturation can be achieved by:
  - Increasing [protein]
  - Increasing [precipitant]
  - Type of precipitant
  - Evaporation
  - Change in temperature





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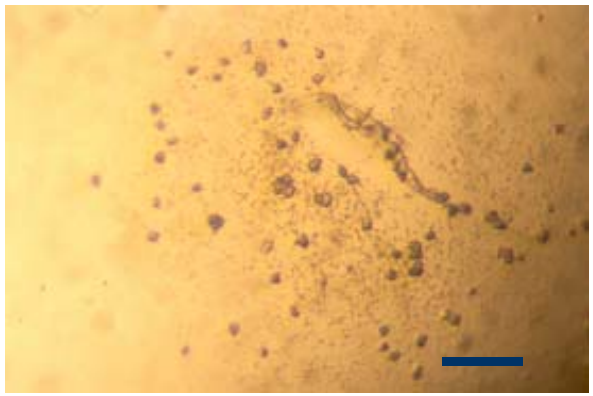
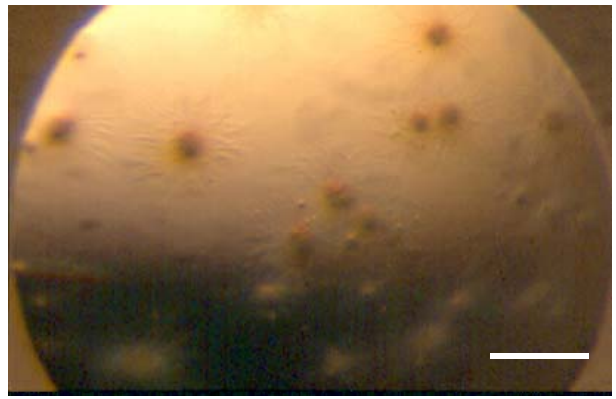
Crystallization – step 2

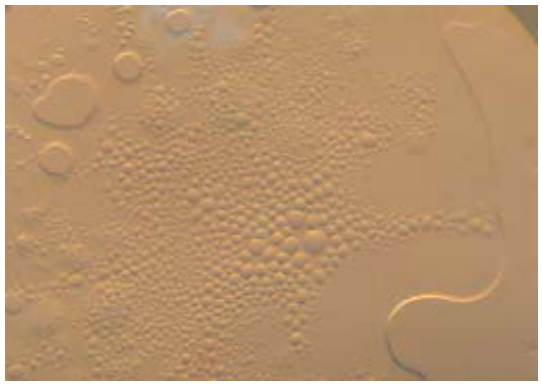
a 

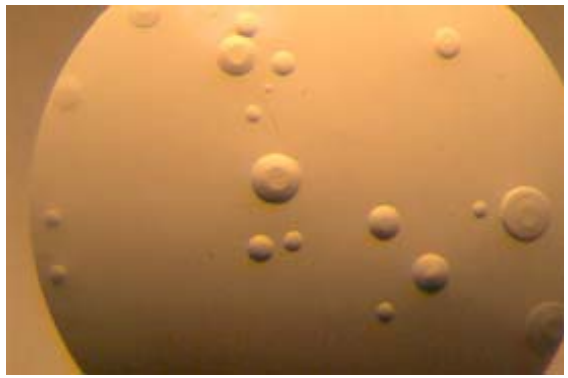
b 

- Gaining control of the crystallization environment
- Controlling rates
  - Nucleation
  - Equilibration
  - Growth

a. Tiny clusters with PEG 400 and CaCl2 Bar is 200 micron. b. spheres with fine hair-like projections radiating from them. Bar is 500 micron

Some images from the screening trials of GLP-1 and Oxm

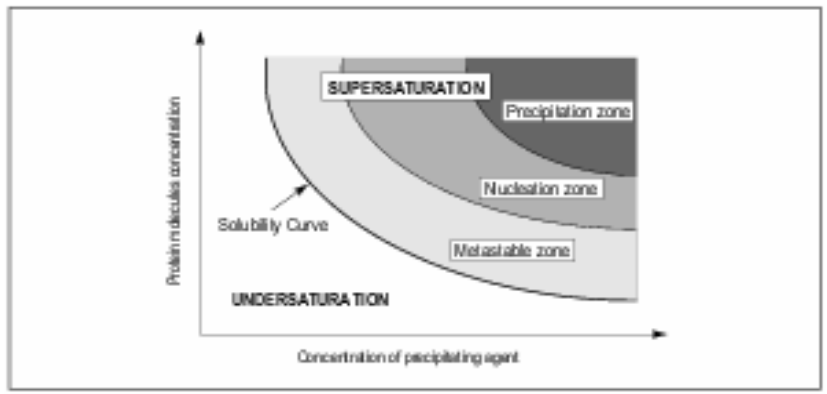




- These images represent phase separation The crystallising agent that produced this result has the potential to lead to crystal formation. However conditions which result in phase separation are usually difficult to optimise

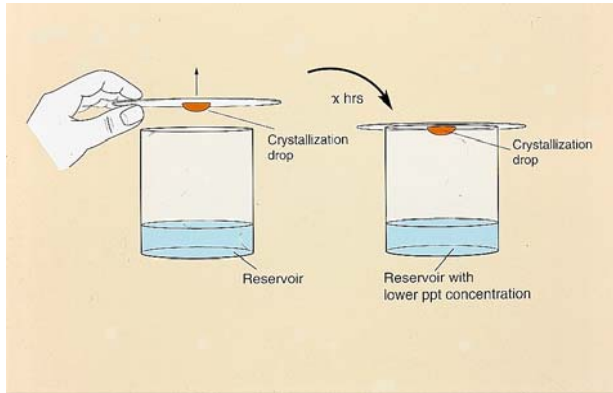
Gaining control of the crystallization environment

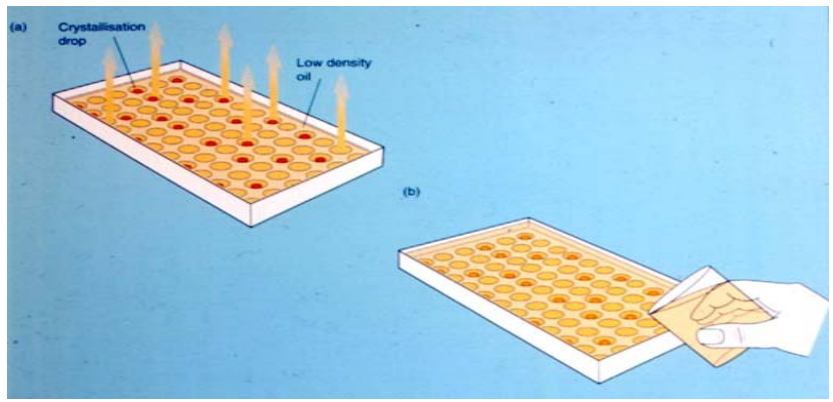
- Fine tuning experimental outcomes
- Altering the drive to equilibrium
  - Too much nucleation?
  - Equilibration and growth
- Dealing with phase separation
  - Temperature
  - pH screens/gradients



Controlling the rate and extent of nucleation

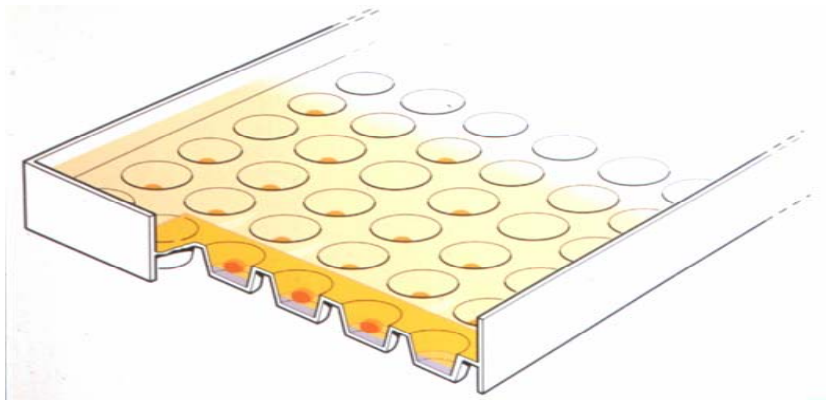
- Manipulating precipitant concentration
  - Separation of nucleation and growth
- Limiting the amount of nucleation
    - Microbatch with controlled evaporation
  - Containerless crystallization in microbatch
- Affecting diffusion rates
  - Microbatch crystallization with gels

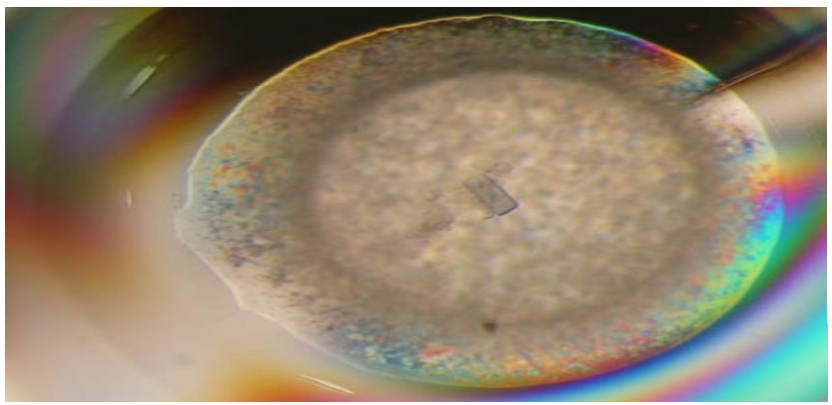




Chayen and Saridakis (2002) Acta Cryst. D58, 921-927

Containerless crystallization

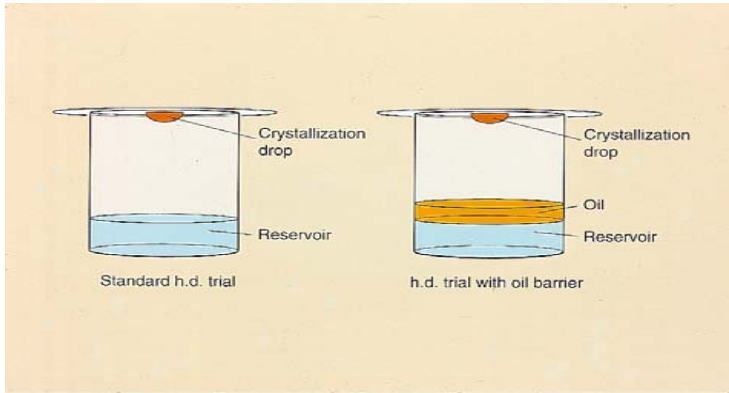


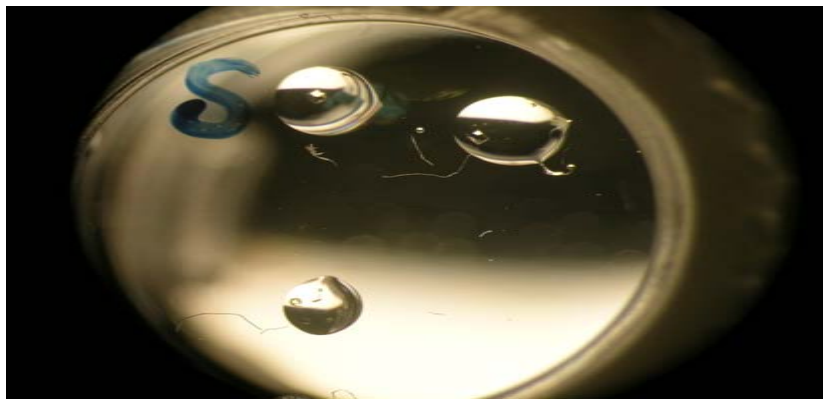


- To reduce contact with the surface so reducing heterogeneous nucleation
- The surface of the Terasaki plate is coated with vaseline and the drop set up under oil

Methods to control rates of equilibration and growth

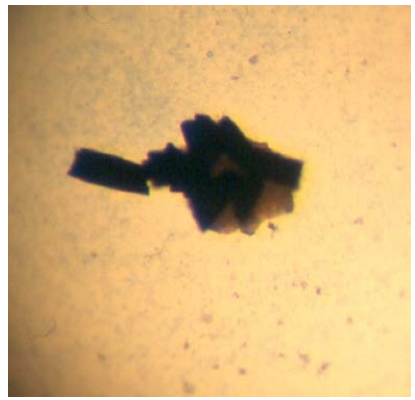
- Oils in crystallization
  - Silicon
  - Paraffin
- Oil barriers in vapour diffusion
- TMOS gels
- The use of nucleants in crystallization

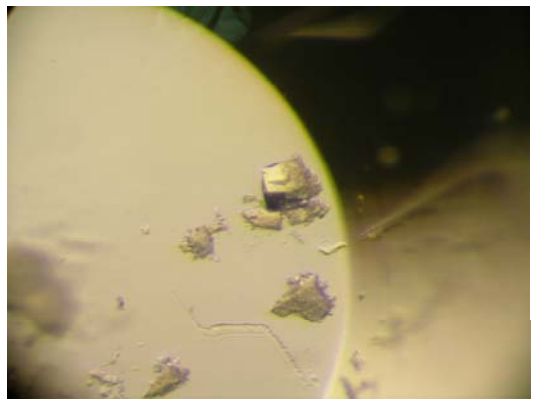




Using nucleants

- What can be used as a nucleant?
- Demonstrated success of porous silicates
- The use of glutaraldehyde as an *in situ* nucleant





When there are no leads

- Inducing nucleation
  - Beyond trial and error
- Moving molecules out of their “comfort zone”:
  - Choice of precipitant(s)
  - Concentration of precipitant(s)
  - Temperature, evaporation
  - The use of nucleants

Towards successful crystallization

- Successfully solublising your protein
  - Choice of detergent, and optimum concentration
- Initial screening – trial and error
  - Fine tuning
- Effectively interpreting leads
  - Design a phase diagram of most promising conditions
- Effectively optimising leads
  - From the phase diagram select optimal conditions, consider additives, consider modifying the method